THE EFFECTS OF CORTISONE ON EXPERIMENTAL COCCIDIOIDOMYCOSIS*

VICTOR D. NEWCOMER, M.D., EDWIN T. WRIGHT, M.D., J. E. TARBET, PH.D., LOUIS H. WINER, M.D. AND THOMAS H. STERNBERG, M.D.

Since the introduction of cortisone and ACTH there has appeared a considerable amount of both clinical and investigative work indicating that these compounds have a profound effect on the host-parasite relationship in various infectious diseases. This relationship has been studied considerably in experimental tuberculosis and to a lesser extent in other infections. The majority of investigators feel that the drugs adversely affect the host response to the parasite. However, there is disagreement as to the mechanism by which this occurs.

The influence of cortisone on vascular tone and capillary permeability is considered by many investigators to be of basic importance as far as the host response is concerned (1–7). Suppression and delay of the inflammatory reaction (7, 8), delay in diapedesis of leukocytes (9), enhanced phagocytic activity (10), have all been cited as resulting from the use of cortisone and ACTH. Finally, there is some evidence that these steroids have no influence on the course of certain infectious diseases (11).

The influence of ACTH and cortisone on the superficial and deep mycoses have not been thoroughly evaluated. Engleman and Krump (12) treated a 27 year old negro male with disseminated coccidioidomycosis with a total of 4.7 gms of cortisone over a 27 day period without altering the clinical course of the disease which terminated fatally. Suttliff and Norman (13) treated three patients with North American blastomycosis. ACTH was administered in a dosage of 25 mgs. every 6 hours for 18 days in one case and for 10 days in the other two. All patients had cutaneous lesions and, in addition, two of them had systemic involvement. A consistent change was noted in the skin lesions manifested by an increased inflammatory reaction and exudation. It was their feeling that there was no indication for the use of ACTH in this disease. Cavallero and Sala (14) evaluated the effect of cortisone on experimental coccidioidomycosis. *Coccidioides immitis* was injected subcutaneously in rats. Post-mortem examination of the control rats showed a local tubercle-like granuloma without any generalized infection, whereas the cortisone treated animals revealed a diffuse infec-

* From the Medical Service, Veterans Administration Center, General Medical and Surgical Hospital, Los Angeles 25, California, and the Division of Dermatology, Department of Medicine, University of California Medical Center, Los Angeles 24, California.

Reviewed by the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions of the authors are the results of their own study and do not necessarily reflect the opinion or the policy of the Veterans Administration.

This research was supported in part by a grant from the Ciba Pharmaceutical Products, Incorporated, Summit, New Jersey.

Read before the thirtenth annual meeting of the Society for Investigative Dermatology, Inc., Chicago, Ill., June 8, 1952.

tion of the spleen and lungs. The production of connective tissue was everywhere greatly inhibited. Kligman, et al. (15), in evaluating *Trichophyton mentagrophytes* infection in guinea pigs, found that the lesions of control and cortisone treated animals were similar. However, in the cortisone treated group there was a delay in the incubation period and the height of the infection occurred at a later period. Healing was prolonged and fungi were present in the skin scrapings for a longer period of time. Biopsy material from the treated and the control groups failed to demonstrate significant differences. Reactivity to the trichophyton test was indistinguishable in the treated and control groups. Upon reinfection of the animals a prolonged healing time of the treated animals was again noted and the inflammatory reaction was of a greater degree. The trichophyton reaction two weeks after reinfection was more prominent in the cortisone treated animals. Jadassohn, et al. (16) infected 22 guinea pigs with *Achorion Quinckeanum*. One half of these received varying doses of cortisone daily. The time of incubation and evolution of the first infection as well as the reinfection were not modified to any appreciable degree by the presence of cortisone.

Tarbet, et al. (17) have recently summarized the interaction of host and parasite response in the mouse upon introduction of the mycelia of *C. immitis* into the peritoneal cavity. They found that the changes observed in the host and parasite were of an orderly progressing nature.

Experimental coccidioidomycosis in mice provides an excellent disease to further clarify the influence of cortisone acetate* on a fungous infection. This report deals with the results of such a study and also considers the in vitro effects of cortisone on *C. immitis*.

MATERIALS AND METHODS

Source of inoculum of C. immitis: Pus aspirated from a subcutaneous abscess of a patient with disseminated coccidioidomycosis was inoculated intraperitoneally into twelve twenty gram CCI male mice. After a two week period the mice were sacrificed by etherization. The mice were immersed completely in a solution of 70% alcohol containing 2½ per cent iodine and allowed to remain in this solution for ten minutes. They were then placed on a dissecting board saturated with full strength Phenolor®. The abdominal wall was opened aseptically and the granulomatous masses in the large omentum were removed with a sterile forceps and scissors and placed in a sterile TenBrock tissue grinder. Twenty cubic centimeters of sterile saline was added to the material ground to a small particle size. Penicillin and streptomycin were added so that each cubic centimeter contained 50 units of each substance. This material was maintained at refrigeration temperature for subsequent use. The number of organisms contained in one cubic centimeter of the suspension was obtained by the usual serial plating technic. This count is based on the production of individual colonies and is therefore only a rough estimation of the true number of viable organisms. It is obvious that one endosporulating spherule may manifest itself as one colony and be counted as one organisms yet contain many hundreds of daughter spherules.

An additional type of inoculum was prepared for use in that phase of this study in which microscopic evaluation was made. It was prepared by the following method: Ten cubic centimeters of Mycophil Broth® was placed in each of six 50 cc. Erlenmyer flasks, and sterilized at 20 pounds of pressure for 15 minutes. They were then inoculated with 0.1 cc of the mouse omentum suspension prepared by the method mentioned above. Ten cubic

* Cortone, Merck and Company, Inc., Rahway, New Jersey, U. S. A.

centimeters of sterile mineral oil was then layered over the broth and the flasks incubated at 37°C. for seven days. Well formed mycelial matts of *C. immitis* had formed at the interface of the mycophile broth and the mineral oil at this time. These matts were removed with a wide mouth pipette and suction with care to avoid the mineral oil. They were placed in a TenBrock tissue grinder, resuspended in sterile normal saline and broken into a fine particle size. Microscopic examination of this material revealed a large number of fragmented mycelial elements. No arthrospores were observed but about 40 per cent of the mycelial elements manifested abortive spherule formation. This was characterized by a rounding of some of the hyphal cells. This effect is generally unavoidable as it results from an inadequate oxygen supply which is produced when mineral oil is layered over the growth media. The use of mineral oil in the preparation of mycelial matts in liquid medias of *C. immitis* is a standard procedure in this laboratory of a precautionary nature. Since the growth of *C. immitis* always occurs at the junction of the media and the mineral oil, the latter serves as a retaining barrier for arthrospores which might be produced earlier than anticipated and become air borne upon manipulating the flasks.

Twenty gram mice, both male and virgin female of the CCI strain were used throughout these experiments. All were maintained on a standard pressed pellet mouse diet. Water was allowed as required. Coritsone was diluted with sterile normal saline and used in various dilutions so that 0.1 cc of the solution contained a suspension of the given dose. This was given intraperitoneally to all groups using a 1 cc tuberculin syringe and a 26 gauge ¼ inch needle. Aseptic technic was used throughout. All injections were given once daily until the completion of the experiment.

Experiment I

The purpose of this experiment was to determine the *in vitro* effects of cortisone acetate upon *C. immitis*. To five of seven 50 cc. Erlenmeyer flasks containing 10 cc. of sterile Mycophil Broth cortisone was added in the following amounts: 0.5, 1.0, 2.0, 3.0, and 4.0 mgs. The remaining two flasks served as controls. All flasks were then inoculated with 0.1 cc. of mouse omentum suspension containing 186,000 spherules per cubic centimeter and were incubated at 37°C. At the end of two weeks they were examined and compared for any effects that cortisone acetate might have produced on the organism.

Experiment II

The purpose of this experiment was to determine if cortisone had a favorable or adverse influence on the course of experimental coccidioidomycoses in mice. In this experiment we were interested in the effects which cortisone might produce when given to mice in that dosage range which is generally used in the treatment of humans rather than the maximum effects which it may be capable of producing. All mice received daily intraperitoneal injections of either saline or cortisone except one group. Injections were continued for 70 days at the end of which time the remaining mice were killed and examined for evidence of infection. Injections were begun four days prior to inoculation with *C. immitis*. On the fourth day all mice except the controls were inoculated intraperitoneally with 0.1 cc. of a saline suspension of mouse omentum containing approximately 84,000 spherules. A total of 168 male mice were used and divided into the following groups:

Group I contained 18 mice. These were inoculated with *C. immitis* and re-

ceived no other injections. This group was to reflect the normal course of the disease.

Group II contained 6 mice and was given daily intraperitoneal injections of saline. This group was to reflect the effects on normal mice of the trauma of daily injections and the effects of saline.

Group III contained 18 mice. These were inoculated with *C. immitis* and received daily intraperitoneal injections of saline in 0.1 cc. amounts. This group was to reflect the traumatic effects of the daily injections and also the effect of the diluent, i.e., normal saline on the course of the disease.

Group IV contained 18 mice. They were inoculated with *C. immitis* and received 0.28 mg. of cortisone daily.

Group V contained 6 mice and received 0.28 mg. of cortisone daily.

Group VI contained 18 mice. They were inoculated with *C. immitis* and received 0.56 mg. of cortisone daily.

Group VII contained 6 mice and received 0.56 mg. of cortisone daily.

Group VIII contained 18 mice. They were inoculated with *C. immitis* and received 1.12 mg. of cortisone daily.

Group IX contained 6 mice and received 1.12 mgs. of cortisone daily.

Group X contained 18 mice. They were inoculated with *C. immitis* and received 1.68 mgs. of cortisone daily.

Group XI contained 6 mice and received 1.68 mgs. of cortisone daily.

Group XII contained 18 mice. They were inoculated with *C. immitis* and received 2.24 mgs. of cortisone daily.

Group XIII contained 12 mice and received 2.24 mgs. of cortisone daily. Twelve mice were used in this group because of preliminary experiments which demonstrated that mice maintained on this higher dosage level occasionally died from intercurrent infection.

Experiment III

The purpose of this experiment was to evaluate microscopically the influence of cortisone therapy on the host-parasite relationship in coccidioidomycosis in the mouse. It was our feeling that if the early developmental stages of coccidioidomycosis were observed under the influence of cortisone from the viewpoint of both host and parasite that any abnormal reactive pattern would be more easily discernible.

It was for this reason hyphal elements of the vegetative phase of *C. immitis* as prepared under "Method and Materials" were used instead of the spherules obtained from the mouse omentum. Developmental changes to spherules and from spherules to maturation and reproduction can be readily observed with the use of hyphal elements. A 0.2 cc. inoculum was used to facilitate the identification of the organism in the early stages of the disease process.

A total of 200 white virgin female mice were used. All mice were inoculated intraperitoneally with *C. immitis*. These mice were placed in the following groups:

Group I contained 20 mice and served as a control for the normal course of the infection.

Group II contained 20 mice and received daily intraperitoneal injections of saline. This group served as a control for the effect of the daily trauma of injections per se and the effect of normal saline on the course of the disease.

Group III contained 40 mice and received 0.56 mg. of cortisone daily.

Group IV contained 40 mice and received 1.12 mgs. of cortisone daily.

Group V contained 40 mice and received 1.68 mgs. of cortisone daily.

Group VI contained 40 mice and received 2.24 mgs. of cortisone daily.

All mice received the above mentioned dosage schedules of saline and cortisone for three days prior to inoculation with *C. immitis.*

Two mice out of groups one and two and four mice out of the remaining groups were sacrificed at the following time intervals after inoculation with *C. immitis:* 4 hours, 8 hours, 24 hours, 2, 3, 4, 5, 8, 10, and 11 days. Those mice remaining continued to receive the daily injections of either cortisone or saline. All mice were sacrificed by etherization and completely immersed in a solution of 70 per cent ethyl alcohol containing 2.5 per cent iodine solution for 15 minutes. They were then placed on a dissection board covered with full strength Phenolor and under aseptic technic the abdomens were opened and examined. Gross examinations were made at this time. Smears of the peritoneal fluids and surfaces as well as tissue sections were taken during the first few days of this experiment. Histological specimens were placed in 10 per cent formaldehyde solution for later microscopic examination. The tissues were prepared by the standard paraffin embedding technic and alternate sections were stained with hematoxylin and eosin and the Hotchkiss-McManus stain (18).

RESULTS

Experiment I

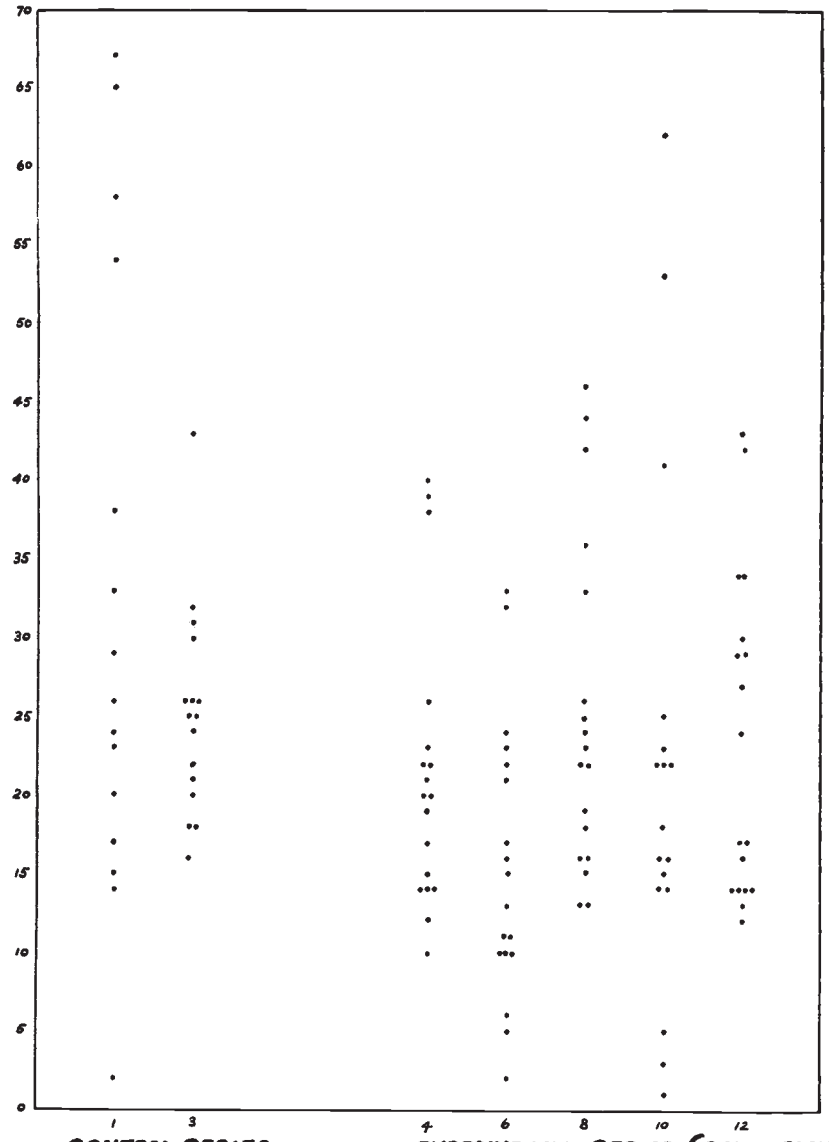
There was approximately the same amount of growth of *C. immitis* observed in the flasks containing the various dilutions of cortisone when compared with the controls. Under the conditions of this experiment there was neither an outstanding inhibiting nor enhancing effect produced by cortisone on the growth of *C. immitis in vitro.*

Experiment II

The results of this experiment are presented in Figure I which shows the number of deaths in each of the groups on the days at which they occurred. This data was subjected to analysis* for the purpose of determining any statistically significant favorable or adverse effects of cortisone. Group III was used as the control. Applying the *t* test and using the 25th day of survival as the end point, it may be assumed that cortisone acetate does have a statistically significant accelerating effect on the infectious process when given daily in doses of 0.56 mgs. However, this is not true if the 26th, 29th, and 30th days are used as the end point. There was no statistically significant effect on the rate of death of mice in all other groups receiving the various dosages of cortisone.

* Eschscholtzia L. Lucia, Ph.D.

FIG. 1. SURVIVAL IN DAYS AFTER INJECTION OF MICE INFECTED WITH COCCIDIOIDES IMMITIS AND GIVEN INCREASING DOSES OF CORTISONE ACETATE



CONTROL SERIES

1. C. immitis only (18 mice) 1/
2. Saline only (6 mice)
   1 death, Trauma of injection
3. C. immitis and Saline (18 mice) 2/

No Deaths
5. Cortisone acetate only 0.28 mgm (6 mice)
7. Cortisone acetate only 0.56 mgm (6 mice)
9. Cortisone acetate only 1.12 mgm (6 mice)
11. Cortisone acetate only 1.68 mgm (6 mice)
13. Cortisone acetate only 2.24 mgm (12 mice)

1/ Three mice killed on 70th day of infection.
2/ One mouse killed on 70th day of infection.

EXPERIMENTAL SERIES (18 MICE EACH)

4. C. immitis and Cortisone acetate 0.28 mgm
6. C. immitis and Cortisone acetate 0.56 mgm
8. C. immitis and Cortisone acetate 1.12 mgm
10. C. immitis and Cortisone acetate 1.68 mgm 3/
12. C. immitis and Cortisone acetate 2.24 mgm

3/ One mouse killed on 70th day of infection.

Experiment III

No visible lesions could be seen upon opening the peritoneal cavity of the various groups of mice until 24 hours. At this time small whitish aggregates approximately 1 mm. in diameter were noted on the parietal peritoneum and floating free between the loops of the intestines. A slight mucoid exudate was present. These findings were more extensive in the cortisone treated animals but no differences could be noted between the various groups receiving different dosages of cortisone.

At 48 hours these findings had become prominent. The nodules had enlarged to a diameter of 1–2 mm. and were slightly adherent to the peritoneal surfaces. The peritoneal exudate had increased and was mucoid and milky in appearance. These observations were present in all of the cortisone treated groups and were of a slightly greater degree than in the control groups.

During the 3rd, 4th, and 5th days in the control animals there were fewer loosely attached nodules and less of them in number observed throughout the peritoneal cavity when compared with earlier observations made in the same groups. However, during this same period of time there was a progressive matting and nodularity of the greater omentum. These same changes were present in the cortisone treated mice and continued to be more extensive.

The nodules were firmly attached in all animals by the 8th, 10th, and 11th days but they were fewer in number. The omentum and adjacent areas were increasingly involved in the disease process and there was a progressive matting of the organs in the peritoneal cavity during these latter days of observation.

The following is a summary of the microscopic observations of smears and tissue sections stained by the hematoxylin-eosin and Hotchkiss-McManus methods.

4 hours. Smears made from the parietal peritoneum and peritoneal fluid from both control, saline, and cortisone treated groups showed an occasional hyphal filament. A cellular exudate was present consisting of polymorphonuclear leukocytes and lymphocytes.

Sections made from omental tissue showed loose aggregates of hyphal elements of *C. immitis* upon and in the close vicinity of this structure. They were infiltrated and surrounded by polymorphonuclear leukocytes and lymphocytes. Dilatation of the capillaries and a sparse infiltrate of polymorphonuclear leukocytes and lymphocytes was present in the tissue and in those areas in close approximation to the aggregated hyphal elements. No microscopic differences could be found between the various groups.

8 hours. Very few hyphal elements could be seen on smears made of the peritoneal exudate and the components of this exudate were predominantly polymorphonuclear and lymphocytic in type.

A considerably larger infiltrate of polymorphonuclear cells and lymphocytes surrounding and infiltrating the aggregated mycelial elements was seen on examination of the tissue sections. An occasional section showed early necrosis of the polymorphonuclear leukocytes and lymphocytes in the center of these ag-

gregates. However, these aggregates were still free upon the omental surface and the adjacent areas contained in general a greater infiltrate of polymorphonuclear leukocytes and lymphocytes than at 4 hours. Throughout these aggregates the fungus was present in the form of mycelial elements and to a lesser extent spherules in chains and clusters. No differences in the fungus were noted when compared with those seen at 4 hours. There were no differences in all the groups when compared with respect to the host response and the fungus.

24 hours. Smears were no longer taken at this time since small visible whitish nodules could be observed in the peritoneal cavity.

Well organized granulomas composed of polymorphonuclear leukocytes and lymphocytes were observed in histological sections from the control animals. Evidence of necrosis of the cellular elements was present in the center of some of these granulomas. The cellular infiltrate around the mycelial elements in the cortisone treated mice was in general of a much greater degree. The granulomas were more edematous and the cellular elements were not as tightly packed. There was also necrosis of the cellular infiltrate in the center of these lesions. Early evidence of fixed tissue proliferation in the adjacent omentum was present in about the same degree in all groups.

C. immitis appeared to be in the same stage of development in all the groups. Hyphal elements and clumps of spherules of the same size as in the earlier groups were observed. Large numbers of organisms were present in all sections studied.

48 hours. Well organized granulomas were present in the control mice. They consisted of a center of necrotic material resulting from the death of polymorphonuclear leukocytes and lymphocytes with a mid-zone of polymorphonuclear leukocytes and lymphocytes. Many of the granulomas appeared to be in the tissue proper and in these areas fibroblastic proliferation was present at the periphery.

The granulomas in the cortisone treated mice had in general the same histological pattern as those in the control groups. However, they were more loosely formed and the zone of polymorphonuclear leukocytes and lymphocytes around the necrotic center was not as distinct and formed irregular patterns which extended into the center. The granulomas appeared more numerous in the cortisone treated groups.

Hyphal elements were still present with evidence of individual cells developing into spherules. All spherules appeared to be of the same size and still in chains and clumps. There appeared to be fewer organisms in the granulomas of the cortisone treated groups.

3 days. The granulomas seen in the control mice maintained their characteristic pattern of central necrosis, a mid-zone of polymorphonuclear leukocytes and lymphocytes and a peripheral zone of fibroblastic cells. The cortisone treated mice had more and larger, loosely knit granulomas. The necrotic element of the granulomas was larger and there was a smaller mid zone of lymphocytes and polymorphonuclear leukocytes. Fibroblastic proliferation was of the same degree.

The organisms in all groups were in the same stage of development. A few hyphae were still present but the majority of organisms were in the form of spherules in small chains of 3–4 cells and in clumps. Few organisms were present

in the sections taken from the controls. The number of organisms varied from section to section in the cortisone treated groups but in general their number was not greater than in the control groups.

4 days. The fibroblastic and fixed tissue elements surrounding the granulomas was well developed in the control mice. However, in the cortisone treated animals the granulomas appeared edematous, larger, and poorly formed. A saw tooth border of lymphocytes and polymorphonuclear cells extending into the necrotic zone was frequently observed. Fibroblastic proliferation was of the same degree in development. The organisms appeared to be more plentiful in the cortisone treated groups and by this time all were in the form of spherules about 20 microns in diameter and were in chains and clumps.

5 days. Very little histological difference could be observed in the tissue response and the organisms when compared to that seen at four days.

8 days. The fibroblastic and fixed tissue elements forming the periphery of the granulomas constituted about one-fourth of their radius in the control groups. The granulomas in the cortisone treated mice were larger. The necrosis was more extensive than in the control groups but the fibroblastic element was of the same degree of development.

In all the groups there was an increase in the size of the spherules but mature organisms (60–80 microns in diameter) were seen only in those mice receiving 0.56 mg. and 2.24 mgs. of cortisone. There was no evidence of reproduction.

10 and 11 days. Fibroblastic proliferation at the periphery composed about one-third of the total area of the granulomas in the control mice. The granulomas were in general larger in the cortisone treated mice and contained large necrotic centers. The peripheral fibroblastic and fixed tissue cellular zone was in general quite well developed but there was considerable irregularity in the width.

Maturation of the spherules was seen in the control groups without evidence of endosporulation. Spherules measured about 60 to 80 microns in diameter. Mature and endosporulating spherules were seen in all the cortisone treated groups. Some of the spherules had ruptured releasing their contents.

DISCUSSION

The *in vitro* evaluation of cortisone against *C. immitis* revealed no marked inhibitory or stimulatory effect on this organism in the dilutions used. It must be pointed out however that these observations are made with regard to the growth of the "vegetative phase" of *C. immitis* and that the results may not be applicable to the growth of the "animal phase" of the organism.

In designing this experiment it was anticipated that cortisone would have a marked adverse effect on the course of *C. immitis* infection in mice. The dosage of cortisone was deliberately set to approximate dosages which might be used in man, the equivalent of 100 to 800 mgs. daily. The results were somewhat surprising since the adverse effect observed was minimal.

Clinically, based on time of death after inoculation, there was not a marked difference between the control and test groups. If the 25th day was used as the end point, the death rate in cortisone Group VI was significantly higher. In other

groups statistically significant changes in mortality rates were not found although there was a trend towards a more rapidly fatal infection in all of the test mice. The greatest adverse effect was in the group of mice receiving the man equivalent of 200 mgs. daily on a weight basis. It is interesting that larger doses apparently were better tolerated. This observation may have clinical significance and it deserves further study. If proven valid, it suggests that the dosage of cortisone producing maximal clinical effect in man is not necessarily the optimal dosage from the standpoint of avoiding secondary bacterial and fungous infections.

An evaluation of the pathologic changes in the test and control groups revealed some consistent differences in pathogenesis. In the control animals the important pathologic changes following inoculation may be summarized as follows:

1. Between 24 and 48 hours miliary nodules were evident grossly, which microscopically were found to be composed of polymorphonuclear leukocytes and lymphocytes surrounding and infiltrating aggregated hyphal elements.

2. By the third to fifth day the nodules had enlarged and granulomas were being formed. These granulomas consisted of necrosis of polymorphonuclear leukocytes and lymphocytes in the center, a zone of viable polymorphonuclear leukocytes and lymphocytes and early fixed tissue proliferation at the periphery.

3. From the fifth to eleventh day the granulomas continued to develop with an increased proliferating, fibroblastic outer zone.

4. The organisms were observed to develop from the hyphal cells to young spherules at 3–5 days and to mature spherules at 10–11 days. No multiplication was noted in the control groups.

The cortisone-treated mice followed essentially the same pathologic pattern except that (1) the immediate influx of polymorphonuclear leukocytes and lymphocytes was of a much greater degree, (2) the granulomas and the necrotic areas in the granulomas were larger and more edematous and (3) maturation and multiplication of the organism occurred earlier in the treated groups.

From these data it is evident that cortisone in the dosage used does not have the profound adverse effect on the host response to *C. immitis* that has been reported for this and other infections in both man and animals (3, 6, 7, 9, 10, 19, 20, 21, 22, 23, 24). One explanation may be the lower dosage of cortisone used in our experiment as compared to that used by most investigators. It is also possible that fungous infections in mice may elicit a different type of host response which is not markedly affected by cortisone.

CONCLUSIONS

1. Cortisone in dilutions of 1:2500 to 1:20,000 had no visible gross effects on the growth of *C. immitis in vitro*.

2. Cortisone had a minimal adverse effect on the longevity of mice infected with *C. immitis* in the dosages used and under the conditions of this study. The adverse effect appeared to be zonal and did not parallel increasing dosages of the drug. A larger series of mice will be required for statistical validation of this observation.

3. Cortisone also had some adverse effect on the early phase of the hosts' response to *C. immitis* as determined by histological examination of the tissues. This consisted of an increase in the quantity of polymorphonuclear and lymphocytic cells resulting in larger granulomas with a larger and more edematous central necrotic component. The peripheral fibroblastic response was apparently unaffected.

4. In mice infected with *C. immitis*, cortisone appeared to produce earlier maturation and multiplication of the fungus.

5. The adverse effect of cortisone in this experiment was much less dramatic than most other reported animal experiments with this and other organisms. An explanation for this variance in results is not readily available.

REFERENCES

1. EBERT, R. H.: In vivo observations on the effect of cortisone on experimental tuberculosis, using the rabbit ear chamber technique. Am. Rev. Tuberc., **65:** 64–74, 1952.
2. MENKIN, V.: Effects of ACTH on the mechanism of increased capillary permeability to trypan blue in inflammation. Am. J. Physiol., **166:** 518–523, 1951.
3. MENKIN, V.: Further studies on mechanism of increased capillary permeability in inflammation with the aid of cortisone and ACTH. Proc. Soc. Exper. Biol. & Med., **77:** 592–597, 1951.
4. MENKIN, V.: Cortisone and mechanism of increased capillary permeability in inflammation. Federation Proc., **10:** 91, 1951.
5. EBERT, R. H.: Changes in connective tissue reaction induced by cortisone—in vivo observations using the rabbit ear chamber technique. J. Clin. Investigation, **30:** 636–637, 1951 (in Soc. Proc.).
6. LURIE, M. B., ZAPPASODI, P., DANNENBERG, A. M., JR., AND LYNCH, E. C.: Constitutional factors in resistance to infection: The effect of cortisone on the pathogenesis of tuberculosis. Abstracts of Papers, 47th Ann. Meeting, Natl. Tuberculosis Assoc., May 14–18, 1951, pp. 4–5.
7. CUMMINGS, M. M., AND HUDGINS, P. C.: The influence of cortisone on experimental tuberculosis. Abstracts of Papers, 47th Ann. Meeting, Natl. Tuberculosis Assoc., May 14–18, 1951, p. 5.
8. MICHAEL, M., JR., AND WHORTON, C. M.: Delay of the early inflammatory response by cortisone. Proc. Soc. Exper. Biol. & Med., **76:** 754–756, 1951.
9. GLASER, R. J., BERRY, J. W., LOEB, L. H., AND WOOD, W. B., JR.: The effect of cortisone on acute bacterial infections. J. Clin. Investigation, **30:** 640–641, 1951 (in Soc. Proc.).
10. LURIE, M. B., ZAPPASODI, P., DANNENBERG, A. M., JR., AND SWARTZ, I. B.: Constitutional factors in resistance to infection: The effect of cortisone on the pathogenesis of tuberculosis. Science, **113:** 234–237, March 2, 1951.
11. LEMAISTRE, C., AND TOMPSETT, R.: The evolution of tuberculosis lesions in the guinea pig during administration of adrenocorticotropic hormone (ACTH) or cortisone. Am. Rev. Tuberc., **64:** 295–306, 1951.
12. ENGLEMAN, E. P., AND KRUPP, M. A.: Clinical experiences with cortisone, Veterans Administration Conference on Cortisone Research. A symposium, August 15 and 16, 1950, pp. 28–36.
13. SUTLIFF, W. D., AND NORMAN, S. L.: Adrenocorticotropic Hormone in Cutaneous and Systemic North American Blastomycosis. Proceedings of Second Clinical ACTH Conference. Vol. II—Therapeutics. John R. Mote, M.D., Editor. The Blakiston Company, Dec. 8, 9, 1950, pp. 381–390.
14. CAVALLERO, C. AND SALA, G.: Cortisone and Infection. Lancet, **1:** 175, 1951.
15. KLIGMAN, A. M., BALDRIDGE, G. D., REBELL, G., AND PILLSBURY, D. M.: The effect of cortisone on the pathologic responses of guinea pigs infected cutaneously with fungi, viruses and bacteria. J. Lab. & Clin. Med., **37:** No. 4, pp. 615–620, 1951.

16. JADASSOHN, W., MACH, R. S., AND NARDIN, J.: The effect of cortisone on experimycosis in the guinea pig. Acta Endocrinol., **6**: 351–355, 1951.
17. TARBET, J. E., WRIGHT, E. T., AND NEWCOMER, V. D.: Experimental coccidioidal granuloma: Developmental stages of sporangia in mice. In press.
18. KLIGMAN, A. M., MESCON, H., AND DELAMATER, E. D.: The Hotchkiss-McManus stain for the histopathologic diagnosis of fungus diseases. Am. J. Clin. Path., **21**: 86–91.
19. SELYE, H.: The influence of STH, ACTH and cortisone upon resistance to infection. Canad. M. S. J., **64**: 489, June 1951.
20. HART, P. D., AND REES, R. J. W.: Enhancing effect of cortisone on tuberculosis in the mouse. The Lancet, **2**: 391–395, 1950.
21. SPAIN, D. M., AND MOLOMUT, N.: Effects of cortisone on the development of tuberculous lesions in guinea pigs and on their modification by streptomycin therapy. Am. Rev. Tuberc., **62**: 337–344, 1950.
22. Leading article: Cortisone and ACTH in tuberculosis. Lancet, **2**: 632–633, 1950.
23. VOLLMER, E. P.: The course of pneumococcal infection in mice during treatment with antibacterial substances and adrenocortical extract. J. Infect. Dis., **88**: 27–31, 1951.

DISCUSSION

DR. EDWARD D. DELAMATER, *Philadelphia, Penn.*: We have done some work along this line and there are a few points I would like to bring out. First of all, species vary widely in their susceptibility to cortisone. Mice may be one of the more resistant groups and the dose of cortisone may have to be higher to get the effects we observed in rabbits and chickens. Perhaps the dosage these workers have been using is not sufficiently high to cause a breakdown of resistance to infection. I would like to ask if they have used much larger doses to check on this point? Secondly, I do not think we should expect an effect upon the organism itself *in vitro*. I think there is only one microorganism in which a steroid compound has been designated as required and that is one of the amoebae. I do not believe that there is any evidence that one of this group of compounds is specifically required for any pathogenic fungi. The effect we see histologically in overdoses apparently fits the early effects we described, in that there is loosening of the cement substance and a freeing of the individual cell and coating of that cell with substances belonging to the hyaluronic acid group. If we remove hyaluronic acid with hyaluronidase there are still other substances of essentially the same nature present. What these substances are we have not found out. We see a tremendous increase in the organisms in the blood stream infections in chickens and syphilis in rabbits; however, I am not convinced that this means there is a direct influence of the cortisone on the organism *in vivo*.

DR. HELEN D. CURTH, *New York, N. Y.*: In substantiation of the paper I also want to report on a clinical observation. I treated three laboratory workers who had handled mice which, for cancer research, were injected with cortisone and other hormones. The girls suffered from extensive fungous infections of their hands. The causative organism was *Trichophyton mentagrophytes* which was traced back to the mice.

DR. J. WALTER WILSON, *Los Angeles, California*: As has been repeatedly pointed out, any one of you may have to undertake the care of a case of acute

coccidioidomycosis, no matter where you live. If you are faced with such a case, withhold ACTH and cortisone and also all antibiotics unless absolutely indicated because of bacterial conditions present. Our present knowledge does not exempt them from interfering with the development of natural mechanisms of immunity.

DR. EDWIN T. WRIGHT, *Los Angeles, Calif. (in closing)*: I wish to thank the discussors for their comments. The dosages used in this study were comparable on a weight basis with those used in man. This accounts for the apparently small amounts of cortisone used. We did not anticipate a marked effect of cortisone on *Coccidioides immitis in vitro*; however, for the sake of completeness, *in vitro* studies were done. It is well known that the main effect of cortisone is not on the parasite but on the host.